GENETICS OF BACTERIAL CHEMOTAXIS

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INTRODUCTION

Many types of motile bacteria are capable of detecting and responding to changes in their environment. Phototactic, chemotactic and thermotactic movements in bacteria are similar to more complex behaviours seen in higher organisms, and constitute useful model systems for investigating the molecular events underlying sensory transduction phenomena. The best-studied of these systems is the chemotactic behaviour of *Escherichia coli* and *Salmonella typhimurium*. Extensive genetic and biochemical analyses of the chemotaxis machinery in these organisms has led to an intriguing picture of how bacteria detect and process sensory information. At the molecular level, the chemotactic apparatus of bacteria has proven to be surprisingly sophisticated, although many of the mechanistic details are still poorly understood. In this chapter I summarize current knowledge in this area, with particular emphasis on the various ways that genetic methods are being used to investigate the chemotactic behaviour of *E. coli* and *S. typhimurium*. This presentation is necessarily brief and rather speculative. More extensive discussion of this subject can be found in a number of recent reviews (Adler, 1975; Berg, 1975; Hazelbauer & Parkinson, 1977; Parkinson, 1977; Goy & Springer, 1978; Koshland, 1978; Macnab, 1978).

Locomotor behaviour

In the absence of chemical stimuli, wild-type *E. coli* and *S. typhimurium* swim about in random walk fashion consisting of smooth translational movements ('runs') and abrupt turning motions ('tumbles') (Berg & Brown, 1972). Tumbling episodes occur approximately once per second and enable the cell to change its swimming direction in an essentially random manner. In the presence of chemical gradients, chemotactic movements are accomplished by altering tumble probability in response to concentration changes encountered as the organism swims (Berg & Brown, 1972; Macnab & Koshland, 1972). For example, whenever an individual

happens to swim toward an attractant source, tumble probability decreases, producing a somewhat longer average run length in the favourable direction. Since bacteria are sufficiently small to be knocked off course by Brownian motion, they spend a great deal of time moving in the 'wrong' direction during a chemotactic response: they must constantly assess their direction of movement relative to the gradient and can only achieve net migration in a biased random walk fashion.

Both runs and tumbles are produced by rotation of the cells' flagellar filaments, which are semi-rigid helical structures that function like propellers (Berg & Anderson, 1973; Silverman & Simon, 1974). A bacterium typically possesses about six flagella emanating from different sites on the cell surface which act together in a bundle at the rear of the cell to generate co-ordinated swimming movements. The hydrodynamic details of bacterial motility are complex, but in essence runs are produced by counter-clockwise rotation of the flagellar filaments, whose helical sense is normally left-handed. (The direction of rotation is defined by viewing along the bundle of filaments toward the cell body.) Clockwise rotation of one or more filaments causes disruption of the bundle and produces a tumbling episode (Macnab & Ornston, 1977). Flagellar rotation can be monitored by tethering a filament to a microscope slide with specific antibodies and observing rotation of the cell body (Silverman & Simon, 1974). Tethered wild-type cells exhibit frequent reversals in rotation which presumably correspond to episodes of runs and tumbles in free-swimming cells (Berg, 1974; Larsen *et al.*, 1974b).

Rotational motion is most likely generated at the base of the flagellum, which is anchored in the cell wall and membranes and has a complex structure in the electron microscope (DePamphilis & Adler, 1971). The flagellar base is composed of approximately ten different proteins (Silverman & Simon, 1977a). It seems likely that many additional proteins required for motility reside in the inner membrane, because the energy source for rotation is known to be derived from an electrochemical gradient of protons across the cytoplasmic membrane (Larsen, Adler, Gargus & Hogg, 1974a). Both electrical (i.e. membrane potential) and chemical (i.e. pH differential) gradients of protons can be harnessed to power flagellar rotation; ATP is not involved (Khan & Macnab, 1980a). Only a few motility-related membrane components have yet been identified biochemically, and it is not at all clear how proton motive force is coupled to flagellar rotation.

Stimulus detection

Chemotactic responses, i.e. changes in the rotational behaviour of the flagella, are triggered by *temporal* changes in attractant or repellent concentrations encountered by the swimming bacteria (Macnab & Koshland, 1972; Brown & Berg, 1974). Concentration measurements are made by means of specific chemoreceptors arrayed either on the external surface of the cytoplasmic membrane or in the periplasmic space between the inner and outer membranes. Receptors for the sugar attractants have been shown to be specific binding proteins. The extent of receptor occupancy therefore provides a measure of ligand concentration in the environment.

The galactose, maltose and ribose receptors are periplasmic binding proteins (Hazelbauer & Adler, 1971; Aksamit & Koshland, 1974; Hazelbauer, 1975); the glucose and other sugar receptors are binding proteins associated with the cytoplasmic membrane (Adler, & Epstein, 1974; Lengeler, 1975). These binding proteins function as specific recognition devices for both chemotaxis and active transport; however, sugar uptake is not required for chemotaxis, so the two processes are clearly different, even though they share a common component (Adler, 1969). The identity of the receptors for the amino acid attractants aspartate and serine is less clear, but they may also be specific binding proteins in the cytoplasmic membrane (Clarke & Koshland, 1979). Competition studies have provided indirect evidence for the existence of a number of repellent receptors as well, including compounds such as fatty acids, alcohols, hydrophobic amino acids, and several divalent cations (Tso & Adler, 1974). None of these repellent receptors has been identified biochemically.

Sensory transduction

In order to respond only to *changes* in receptor occupancy, and not to absolute chemical concentrations, the bacteria must have a means of adapting their response machinery to static levels of attractants and repellents. The existence of such an adaptation system has been convincingly demonstrated by subjecting cells to rapid, isotropic changes in attractant or repellent concentration. Attractant increases and repellent decreases suppress clockwise (CW) rotation, whereas stimuli of opposite sign tend to enhance CW rotation (Macnab & Koshland, 1972; Larsen *et al.*, 1974b; Berg and Tedesco, 1975; Spudich & Koshland, 1975). In the absence of further stimuli, these responses persist for a period of time that is directly

related to the magnitude of the change in receptor occupancy. The return to a pre-stimulus behavioural pattern marks the completion of the adaptation process.

These sorts of temporal stimulation studies have served to define two phases to the chemotactic response. Upon encountering a change in attractant or repellent concentration, the cell undergoes an immediate change in flagellar behaviour. This constitutes the excitation phase of the response, during which the level of some sort of internal tumble-controlling signal is altered by chemotactic stimuli. During the adaptation phase of the response, this signal is returned to its pre-stimulus value. The nature of the tumble-controlling signal(s) is not known, but at some point sensory inputs from different receptors must be integrated by the response machinery, because the cell is able to sum multiple or conflicting stimuli algebraically (Tsang, Macnab & Koshland, 1973; Adler & Tso, 1974; Berg & Tedesco, 1975; Spudich & Koshland, 1975). Thus different receptor signals are functionally equivalent. Moreover, all receptors appear to use a common adaptation system because response times to combinations of stimuli are essentially additive.

GENETIC ANALYSIS OF CHEMOTAXIS

The chemotactic machinery of *E. coli* and *S. typhimurium* constitutes a network of signalling elements through which sensory information about the chemical environment is transmitted from chemoreceptors to flagella. These informational pathways are amenable to genetic dissection in much the same manner as more conventional biochemical pathways. The only substantial difference is that defects in biochemical pathways usually lead to accumulation of a discrete chemical intermediate which can be identified and used to reconstruct the sequence of reaction steps. The sequence of signalling steps in the chemotactic pathway is more difficult to establish because very few of the informational intermediates have been identified. Nevertheless, a probable pathway of information flow can be constructed by examining mutants with various sorts of chemotaxis defects. Since there are many chemoreceptors which ultimately transmit signals through a common transduction mechanism, the severity of a mutant phenotype should reflect the position of the defective component in the convergent signalling pathway.

In *E. coli*, approximately 50 loci are known to be required either

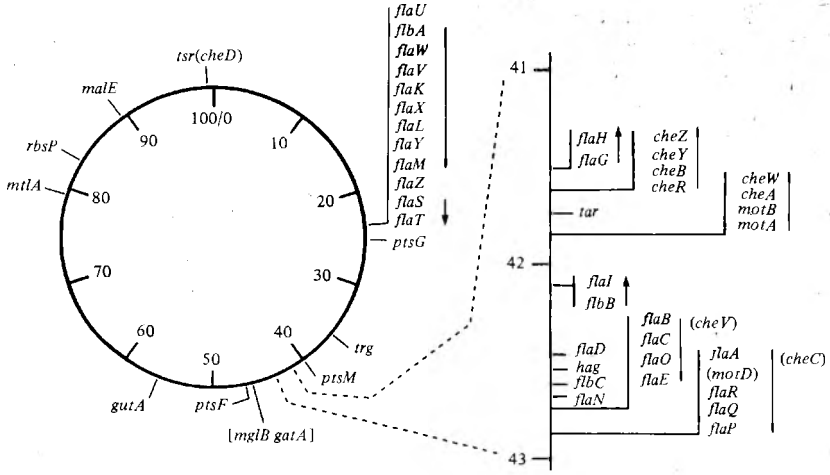

Fig. 1. Genetic map of chemotaxis loci in *E. coli*. Numbers within the circle indicate map position in minutes on the *E. coli* chromosome. In cases where a locus can give rise to more than one type of mutant defect, the primary or null type is shown first, followed by special types shown in parentheses. Arrows above the genes indicate the direction and extent of transcriptional units.

Locus	*Mutant phenotype*	*Probable function*
gatA, gutA, malE, mglB, mtlA, ptsF, ptsG, ptsM, rbsP	lack responses to a limited set of related compounds	chemoreception
tar, tsr, trg	multiple response defects	signalling
cheA, B, C, D, R, V, W, Y, Z	motile, but generally nonchemotactic	regulation of flagellar rotation
fla, flb (27 genes)	nonflagellate	structure and assembly of flagella
motA, motB	flagellar paralysis	energize rotation (?)
hag	filament defects	filament protein

for motility or for chemotaxis (Fig. 1). Mutational alterations in these genes can lead to four basic types of chemotaxis defects. Chemoreceptor mutants, which define the input end of the signalling pathway, have normal motility and lack responses to only a few structurally related compounds. Although there is physiological evidence for at least 20 different chemoreceptor species, many of them, notably the amino acid and repellent receptors, have not yet been identified by mutation. The output end of the chemotaxis machinery is defined by motility mutants, which are either unable to synthesize flagella (*fla* mutants) or which have aberrant filaments (*hag* mutants) or paralysed flagella that cannot rotate (*mot* mutants). Recent reviews on chemoreception (Hazelbauer &

Table 1. *Chemotaxis genes involved in signalling and information processing*

Locus	Gene product		Mutant phenotype			
	Size[a]	Location[b]	Flagellar rotation	Response to temporal stimuli[c]		
				Serine	Aspartate	Ribose
tar	~60 000	cyt. memb.	normal	+	−	+
tsr	~65 000	cyt. memb.	normal	−	+	+
trg	~55 000	cyt. memb.	normal	+	+	−
cheA	78 000 69 000	cytoplasm* cytoplasm	CCW	−	−	−
cheB	38 000	cytoplasm*	*CW*	±	(−)	±
cheC	?	?	*CCW or CW*	±	±	±
cheD	(65 000)	(cyt. memb.)	CCW	(−)	(−)	(−)
cheR	28 000	cytoplasm*				
cheV	?	?	CCW or CW	±	±	±
cheW	15 000	cytoplasm	CCW	−	−	−
cheY	11 000	cytoplasm	CCW	(−)	(−)	(−)
cheZ	28 000	cytoplasm	CW	±	+	+

[a] Approximate molecular weight of proteins based on sodium dodecyl sulphate-polyacrylamide gel electrophoresis; data are from Silverman & Simon, 1977b, 1977c and from Parkinson *et al.*, unpublished.

[b] Data of Ridgway, Silverman & Simon, 1977; asterisk indicates that a portion of the gene product was also found in the cytoplasmic membrane fraction.

[c] Ability of sudden changes in attractant concentration to elicit changes in flagellar rotation. Responses in brackets are weak or equivocal. Data of Parkinson, 1974, 1976 and unpublished; Springer, Goy & Adler, 1977b; Hazelbauer & Harayama, 1979.

Parkinson, 1977) and motility (Iino, 1977) should be consulted for further discussion of these topics.

Two groups of mutants, whose properties are summarized in Table 1, appear to define transduction elements that link the chemoreceptors and flagella. So-called signalling mutants (*tar, tsr, trg*) have normal motility, but lack a number of responses that are initiated by different chemoreceptors (Ordal & Adler, 1974; Reader *et al.*, 1979). Each signalling element therefore seems to handle inputs from a subset of chemoreceptors. Generally non-chemotactic (*che*) mutants (Armstrong, Adler & Dahl, 1967) are unable to carry out effective chemotactic responses to any compounds and must define transduction components common to all of the signalling pathways. Although capable of flagellar rotation, all *che* mutants exhibit aberrant swimming patterns characterized by too few or too frequent tumbling episodes. No *che* mutants with normal tumbling behaviour have been isolated, suggesting that all *che* functions are

somehow involved in generating or regulating reversals in the direction of flagellar rotation. Many *che* strains are still capable of responding to chemotactic stimuli in temporal assays, but have high response thresholds or altered adaptation behaviour.

The central machinery of chemotaxis, as defined by signalling and *che* mutants, appears to be quite similar in *E. coli* and *S. typhimurium*. Nine *che* loci have been identified in both organisms (Armstrong & Adler, 1969a; Aswad & Koshland, 1975a; Collins & Stocker, 1976; Parkinson, 1976, 1978; Silverman & Simon, 1977b; Warrick, Taylor & Koshland, 1977), and the correspondence between *che* functions in the two species has been established by complementation analysis in which F-prime elements carrying *E. coli che* genes were used to correct *che* defects in *Salmonella* recipients (DeFranco, Parkinson & Koshland, 1979). Direct evidence was obtained for correspondence of seven *che* functions in the two organisms, and a uniform nomenclature of these seven loci has been adopted (see Table 1). Mutants of the *cheV* class have not yet been examined by interspecies complementation tests, but have similar phenotypes and map positions in both organisms and are tentatively assumed to be homologous (Warrick *et al.*, 1977; Parkinson, unpublished observations). Thus far *cheD* mutants are only known in *E. coli* and *cheS* mutants only in *S. typhimurium*. Both types of mutants are quite rare and probably arise through highly specific alterations of chemotaxis-related functions (Parkinson, 1976, 1980; Warrick *et al.*, 1977). Three types of signalling mutants have been isolated in *E. coli: tar* mutants are defective in aspartate and maltose taxis (Reader *et al.*, 1979); *tsr* mutants are defective in serine taxis and in a variety of repellent responses (Hazelbauer, Mesibov & Adler, 1969; Tso & Adler, 1974); *trg* mutants are defective in ribose and galactose responses (Ordal & Adler, 1974; Hazelbauer & Harayama, 1979). Only *trg* mutants have thus far been isolated in *Salmonella* (M. Fahnestock, cited in Strange & Koshland, 1976); however, biochemical and physiological evidence for signallers comparable to the *tar* and *tsr* products has been obtained in this organism. Much of the following discussion will draw primarily on the work in *E. coli*, although most of the conclusions will undoubtedly apply to *Salmonella* as well.

As shown in Table 1, the gene products associated with most of the signalling and *che* functions have been identified. The *tar*, *tsr*, *trg* and *cheD* products are cytoplasmic membrane proteins, whereas the *cheA*, *cheB*, *cheR*, *cheW*, *cheY* and *cheZ* products are primarily

located in the cytoplasm (Ridgway *et al.*, 1977). A portion of the *cheB* and *cheR* proteins as well as the larger of the two *cheA* proteins, are also found associated with the cytoplasmic membrane. With the exception of *trg*, these product identifications were made by utilizing plasmids or specialized λ transducing phages to synthesize radio-labelled chemotaxis proteins in minicells or in heavily UV-irradiated whole cells (Silverman & Simon, 1977b; Matsumura, Silverman & Simon, 1977). The genetic content of different plasmids or phages, established by complementation analysis, could be compared with the proteins made by these strains to establish the identity of each gene product. Although λ transducing phage carrying the *cheC* and *cheV* loci have been constructed (Komeda, Shimada & Iino, 1977; Kondoh, 1977), these gene products have not yet been observed by these methods, perhaps because their promoters are less active than other *che* promoters under these experimental conditions.

The probable roles of the signalling and *che* components in chemotaxis are summarized in the two sections below on excitation and adaptation. Many of the transduction elements are important in both processes, but in order to simplify this discussion, the two response phases will be treated separately. Because a great deal of physiological, biochemical and genetic evidence is currently available, interpretive models are presented as a means of summarizing these diverse studies.

EXCITATION

Components involved in the excitation phase of the chemotactic response are shown in Fig. 2. The excitation pathway is clearly convergent, with many inputs at the receptor end but only a single output at the flagellum. The summation of conflicting signals can probably occur at several different points in the pathway depending on the applied stimuli. For example, aspartate and maltose stimuli, which are both handled by the *tar* signaller, must be integrated by the *tar* product. However, aspartate and ribose stimuli, which are channelled through different signallers, must be integrated by some common component later in the pathway. The eventual target of all incoming signals must be the flagellum, which appears to be controlled by some sort of switch.

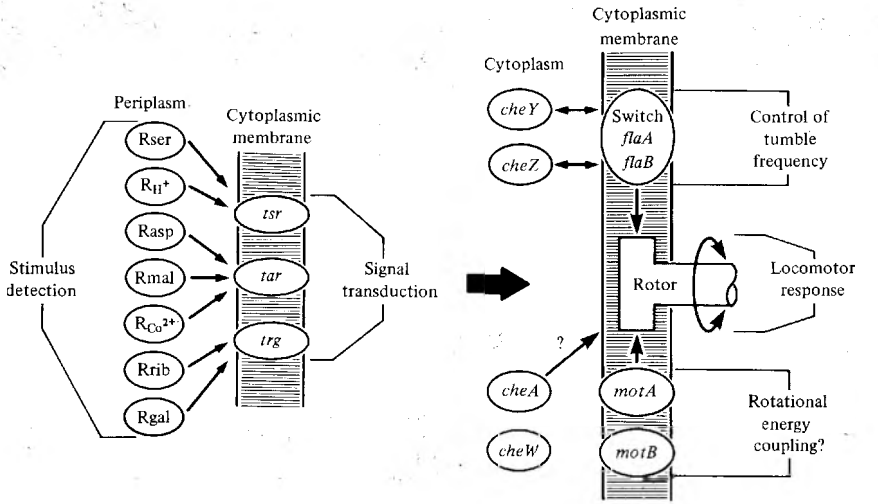

Fig. 2. Components of the excitation system. Occupied chemoreceptors are thought to interact with membrane proteins that in turn generate signals to control the direction of flagellar rotation. The nature of the excitatory signal(s) is unknown.

The flagellar switch

The *cheC* and *cheV* gene products may be the flagellar target site for incoming receptor signals. Mutants of the *cheC* and *cheV* classes are quite rare relative to other *che* strains. In fact, many of the available *cheC* and *cheV* mutations in *E. coli* were obtained as suppressors of other *che* defects during reversion analyses (discussed in a later section). In transductional crosses, *cheC* and *cheV* mutations map in the vicinity of several *fla* operons (Armstrong & Adler, 1969b; Silverman & Simon, 1973; Parkinson, 1976 and unpublished data), indicating that *cheC* and *cheV* mutants might have specific flagellar defects. The relationship of *cheC* and *cheV* to *fla* functions has been investigated with the aid of a specialized λ transducing phage that carries the *cheC–cheV* region of the *E. coli* chromosome (Fig. 3) (Parkinson, Parker & Houts, unpublished data). Deletions of the parental phage were obtained by selecting particles resistant to inactivation by chelating agents. These deletions were then used to construct a fine-structure map of the region by complementation and recombination analyses. As shown in Fig. 3, *cheC* mutations map at the *flaA* locus and *cheV* mutations at the *flaB* locus. These *fla* loci, which are essential for synthesis or assembly of flagella, can evidently be altered in subtle ways by *cheC* or *cheV* mutations to

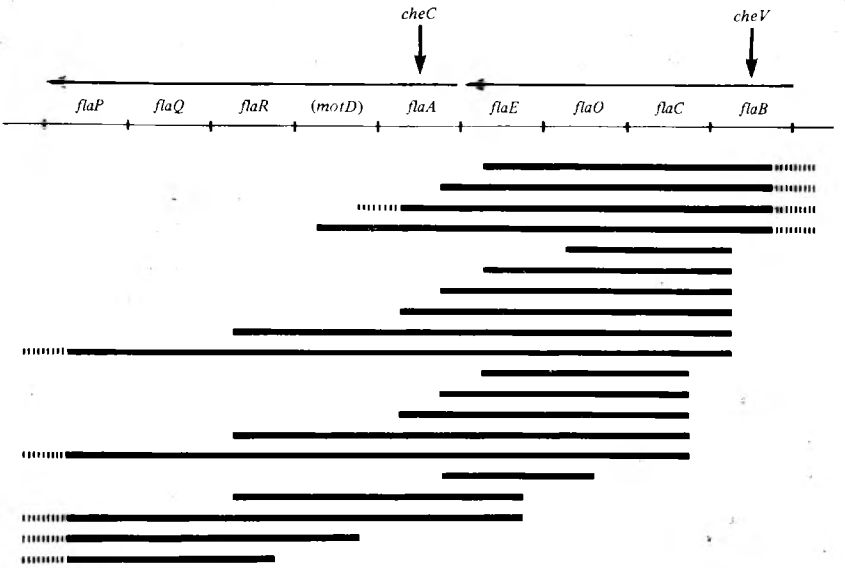

Fig. 3. Localization of *cheC* and *cheV* mutations by deletion mapping. Deletions were obtained by chelating agent inactivation (Parkinson & Huskey, 1971) of *λfla36*, a plaque-forming transducing phage that carries the *flaA* and *flaB* operons (Komeda *et al.*, 1977). Deleted phages were crossed to representative *fla* and *che* strains, as described by Parkinson (1978), to map *cheC* and *cheV* mutations (Parkinson *et al.*, unpublished data).

permit flagellar assembly while interfering with normal rotational behaviour, thereby causing a defect in chemotaxis.

Unlike other *che* loci, mutations at the *cheC* and *cheV* loci can result in either excessively high or low tumbling rates (Warrick *et al.*, 1977; Rubik & Koshland, 1978; Parkinson *et al.*, unpublished data). The fact that specific *flaA* (i.e., *cheC*) and *flaB* (i.e., *cheV*) defects can lead to predominately CW or counter-clockwise (CCW) rotation suggests that these flagellar proteins play a role in controlling the direction of flagellar rotation. The *flaA* and *flaB* products may constitute a 'switch' that is intimately associated with the flagellar basal complex and determines its direction of rotation. In the wild-type cell this switch is set to produce CW and CCW rotation with equal probabilities, but mutations of the right sort can introduce a bias in either direction.

The switch is thought to receive signals generated by the chemoreceptors during excitation. The switch also must respond to signals that set the spontaneous tumbling rate of the cell. The nature of these signals is poorly understood; however, genetic evidence discussed in later sections suggests that the two signal types may not

be identical. In any case, the chemotaxis defects of *cheC* and *cheV* mutants are probably due to an inability to respond properly to these controlling signals. For example, a mutant with CW bias in the switch would require a correspondingly large CCW signal in order to initiate a change in flagellar rotation. Physiological studies of both CW and CCW switch mutants demonstrate that they can still respond to stimuli, but with higher than normal thresholds (Parkinson, 1976 and unpublished data; Rubik & Koshland, 1978). In fact, some switch mutants with only slight alterations in spontaneous tumbling rate exhibit considerable chemotactic ability under certain assay conditions (Parkinson *et al.*, unpublished data).

Regulation of tumbling rates

The *cheY* and *cheZ* products appear to play a role in regulating the rotational behaviour of the cell, probably by interacting directly with the flagellar switch. Mutants with *cheY* and *cheZ* defects resemble switch mutants in several respects: *cheY* strains exhibit extreme CCW bias in their flagellar behaviour, whereas *cheZ* strains have an extreme CW bias; both types of mutant can still respond to chemotactic stimuli, but with high thresholds (Parkinson, 1974, 1976, 1978; unpublished data). Unlike switch mutants, however, *cheY* and *cheZ* defects can arise through nonsense mutations or deletions which eliminate gene function entirely. Thus neither *cheY* nor *cheZ* function is essential for flagellar formation as the switch products are, but they do play a role in setting the cell's spontaneous tumbling rate.

The functional relationship between the *cheY* and *cheZ* products and the flagellar switch has been investigated by searching for direct interactions among the various gene products. Reversion analysis is a powerful method for detecting such interactions, particularly weak ones that would be difficult to observe by conventional biochemical methods (Parkinson & Parker, 1979). The reasoning behind this approach is that when two gene products interact, defects in one can often be alleviated by a compensating alteration of the other. This strategy has been applied to the *che* system by selecting revertants of *cheY* or *cheZ* mutants and then examining those revertants for the presence of suppressor mutations in other chemotaxis genes. When the original mutants carried missense mutations in *cheY* or *cheZ*, many of the chemotactic revertants acquired compensating mutations at the *cheC* and *cheV* loci (Parkinson *et al.*, unpublished data).

The nature of the interactions between the products of *cheY* and *cheZ* and the switch components has been deduced by studying the specificity of these suppression effects. In the first place, not all *cheZ* or *cheY* defects can be suppressed. For example, nonsense mutations, which do not make a complete gene product, cannot be corrected by any sort of switch mutation. This implies that the mutant *cheY* and *cheZ* gene products even though they are aberrant are still required for suppression to occur. Moreover, a mutant suppressed by one switch mutation may not be corrected by another. Finally, suppressors of *cheY* defects cannot correct *cheZ* defects and *vice versa*. These results indicate that the *cheY* and *cheZ* products probably interact directly with the switch proteins.

These interactions could play a role in controlling the cell's spontaneous tumbling rate. For example, the switch may have two alternative states or configurations, one that causes CCW rotation, and one that causes CW rotation. The spontaneous tumbling frequency is presumably determined by the rate at which transitions occur from one switch state to the other. The *cheY* and *cheZ* products could be involved in initiating these transitions or in stabilizing one form or the other of the switch. As a general rule, *cheZ* mutants, which are tumbly or excessively CW, are suppressed by CCW switch mutants; the converse is true for *cheY* mutants. It is possible that in the CCW state the switch has affinity only for *cheZ* protein, not *cheY* protein; in the CW state, the switch may bind *cheY* protein, but not *cheZ* protein.

If this model is true, the relative amount or activity of the *cheY* and *cheZ* proteins should influence the swimming behaviour of the cell. It seems unlikely that these proteins are directly involved in excitation, however they could influence the cell's responsiveness by altering the threshold characteristics of the switch. Moreover, the swimming patterns of other *che* strains, in particular *cheB* and *cheR* mutants (see the section on adaptation), may be due primarily to their effects on the *cheY* and *cheZ* proteins. One way to test these ideas is to alter the normal stoichiometry of the system by selectively changing the number of copies of *cheY* or *cheZ* genes in the cell. This can be readily accomplished with λ transducing phages, and such experiments are presently in progress.

Generation of CW rotation

Mutants defective in *cheA* or *cheW* function have a complete CCW bias in flagellar rotation and are unable to respond to even the very

strongest sorts of CW stimuli (Warrick *et al.*, 1977; Smith & Parkinson, unpublished data). Reversion analyses indicate that *cheA* may interact with the switch and other flagellar components (R. Smith, personal communication). (*cheW* has not yet been examined.) It seems that *cheA* (and perhaps *cheW*) function is required to activate or potentiate the flagellar motor for CW rotation. It is known for example that CCW rotation is possible at lower values of the proton motive force than is CW rotation (Kahn & Macnab, 1980b). It may be that *cheA* and *cheW* are involved in coupling proton motive force to the switch or motor. In this regard it is interesting to note that the *cheA* and *cheW* genes form an operon with the two *mot* genes, which are probably involved in energizing flagellar rotation (Silverman & Simon, 1976).

The *cheA* locus directs the synthesis of two polypeptides, designated p[*cheA*]$_S$ and p[*cheA*]$_L$ (Silverman & Simon, 1977b). These two products are identical in sequence except that p[*cheA*]$_L$, the larger of the two, has an additional 90 or so amino acid residues at its amino terminal end. Examination of the polypeptides produced by various nonsense mutants of *cheA* indicates that both proteins are made from the same coding sequence and are translated in the same reading frame, but from different start sites (Smith & Parkinson, 1980). The *cheA* locus thus appears to be the first example of overlapping genes in bacteria because complementation tests indicate that both proteins are probably needed for chemotaxis, but the purpose in making two similar proteins in this manner is not yet clear. One attractive possibility is that p[*cheA*]$_L$ and p[*cheA*]$_S$ interact with different components of the chemotaxis machinery. For example, the amino terminal sequence of p[*cheA*]$_L$ may enable it to enter the cytoplasmic membrane, whereas p[*cheA*]$_S$ is restricted to the soluble portions of the cell.

Signalling

The *tar*, *trg* and *tsr* gene products are inner membrane proteins that appear to be required for the generation of chemoreceptor signals. Each of these signalling elements handles inputs from a set of chemoreceptors. Presumably, occupied receptor proteins are capable of interacting with the appropriate signaller species to elicit tumble-controlling signals. These signals are assumed to act on the flagellar switch; however, their nature and mode of transmission are not yet understood.

Mutants of the *cheD* class provide a handle of sorts on the

signalling problem. These mutants are relatively rare and prove to be completely dominant to wild-type in complementation tests, implying that they produce an altered gene product that actively interferes with chemotaxis. Genetic studies strongly indicate that the *cheD* phenotype is generated by a special alteration of the *tsr* signaller (Parkinson, 1980). For example, *cheD* and *tsr* mutations are very tightly linked in a region of the genetic map that contains no other known chemotaxis loci. Moreover, the inhibitory product made by *cheD* strains can be inactivated by *tsr* lesions, suggesting that the inhibitor is an altered form of the *tsr* product. Both *tsr* and *cheD* defects could conceivably arise by changes in the same gene product. Null mutations such as deletions, which completely abolish function, would lead to a Tsr$^-$ condition, whereas special missense mutations might convert the *tsr* protein into an inhibitor of chemotaxis. Since *cheD* mutants have low tumbling frequencies, they may synthesize a *tsr* signaller that is somehow locked in the CCW signalling mode. This model predicts that it should be possible to restore chemotaxis in *cheD* strains by selectively modifying the normal target of *tsr* signals so that it is no longer inhibited by aberrant signals due to the *cheD* defect.

External suppressors of *cheD* mutations have been obtained by isolating pseudorevertants from strains with two copies of the mutant *cheD* locus (Parkinson, unpublished data). Because *cheD* defects are dominant, two independent mutations, one in each *cheD* copy, are required to produce either true revertants or Tsr$^-$ pseudorevertants of *cheD* diploids and these reversion types should prove to be relatively rare in this selection, which favours detection of external suppressors that can arise in a single step. These studies revealed that mutations at the *cheB*, *cheZ*, *flaA* and *flaB* loci were capable of suppressing *cheD* defects. All such suppressors exhibit considerable CW bias of flagellar rotation in the absence of the *cheD* lesion; in combination with *cheD*, flagellar rotation approximates wild-type behaviour. None of these interactions appears to be allele-specific, suggesting that restoration of a normal tumbling rate, by whatever means, is sufficient to restore chemotactic ability in *cheD* strains. This result implies that the excitation and adaptation machinery is still intact in *cheD* mutants, but cannot function properly unless the tumbling rate is increased.

If the *cheD* model is valid, the failure to obtain specific target mutants could mean that all receptor signals are qualitatively identical and therefore the component that receives those signals is

unable to discriminate among them. Thus it may not be possible for the cell to ignore *cheD* signals and still detect other signal inputs; the only recourse for reversion is to reset the spontaneous tumbling frequency so that *cheD* signals no longer 'drown out' the other channels. A second conclusion from these studies is that the interaction between a signaller and its target may not be a direct protein–protein interaction. If the interaction were direct, it seems unlikely that all signallers would appear identical to the target since the *tar*, *tsr* and *trg* proteins are clearly not identical to one another. Two possible signal types are consistent with these findings. The signal could be a cytoplasmic molecule or ion whose concentration is regulated by the transduction machinery. Alternatively, the signallers might control changes in the cell's membrane potential to initiate flagellar responses.

ADAPTATION

A variety of studies, principally by Adler and his co-workers (reviewed by Springer, Goy & Adler, 1979), have shown that sensory adaptation is accompanied by a net change in the level of methylation of several inner membrane proteins known as 'methyl-accepting chemotaxis proteins' or MCPs (Kort, Goy, Larsen & Adler, 1975). The changes in MCP methylation elicited by various chemotactic stimuli are illustrated in Fig. 4. Tumble-suppressing stimuli such as attractant increases produce a net increase in MCP methylation; tumble-enhancing stimuli such as repellent increases cause a net decrease in methylation level. The kinetics of methylation or demethylation following application of a chemotactic stimulus are quite similar to the time-course of adaptation, suggesting that methylation–demethylation of MCP plays a causative role in the adaptation process (Goy, Springer & Adler, 1977).

The methyl groups on MCP are derived from methionine, via S-adenosylmethionine (SAM), and are attached as methyl esters to glutamic acid residues (Kleene, Toews & Adler, 1977; Van der Werf & Koshland, 1977). Removal of the methyl groups is readily accomplished by hydrolysis, yielding methanol (Toews & Adler, 1979). Each MCP appears to possess several methyl-accepting sites per molecule (Chelsky & Dahlquist, 1980; DeFranco & Koshland, 1980; Engström & Hazelbauer, 1980). Following stimulation, methyl groups may be added to or removed from these sites in a

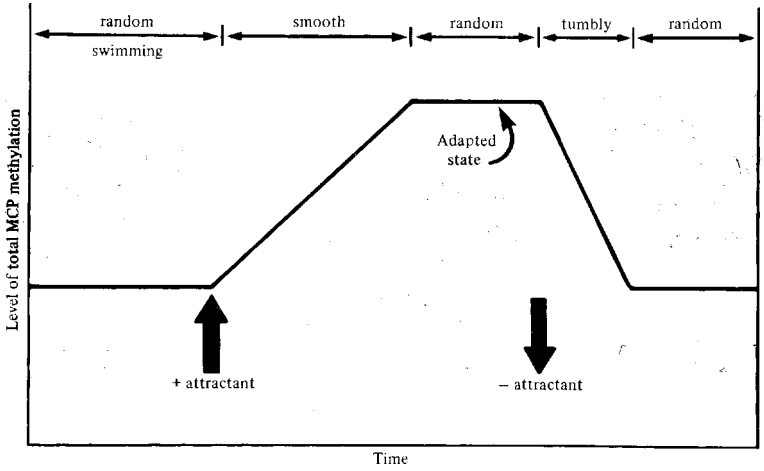

Fig. 4. Effects of chemotactic stimuli on swimming pattern and MCP methylation. Evidence for this scheme is summarized by Springer *et al.* (1979).

specific sequence. Cells deprived of methyl donors, either methionine or SAM, are still capable of responding to certain types of chemotactic stimuli, but are unable to adapt to those stimuli (Armstrong, 1972a, b; Aswad & Koshland, 1974, 1975b; Springer *et al.*, 1975; Springer, Goy & Adler, 1977a). Mutants lacking *cheR* function, discussed below, have a similar defect, indicating that MCP methylation is essential for adaptation, but apparently is not crucial for excitation.

Methylation substrates

Three different MCP structural genes have been identified by mutation, but there is reason to suspect that at least one additional MCP species may exist (Koiwai, Minoshima & Hayashi, 1980). MCPI activity is missing in *tsr* mutants; MCPII is missing in *tar* mutants; and MCPIII is missing in *trg* mutants (Springer *et al.*, 1977b; Silverman & Simon, 1977c; Kondoh, Ball & Adler, 1979). Lambda transducing phages carrying the *tsr* or *tar* loci synthesize methyl-accepting proteins upon infection that appear identical to MCPI and MCPII, respectively (Silverman & Simon, 1977c). These findings indicate that the *tsr* and *tar* gene products (and presumably the *trg* product as well) are MCPs involved in sensory adaptation. Since these gene products are also required for signal production during the excitation phase of the chemotactic response, they must

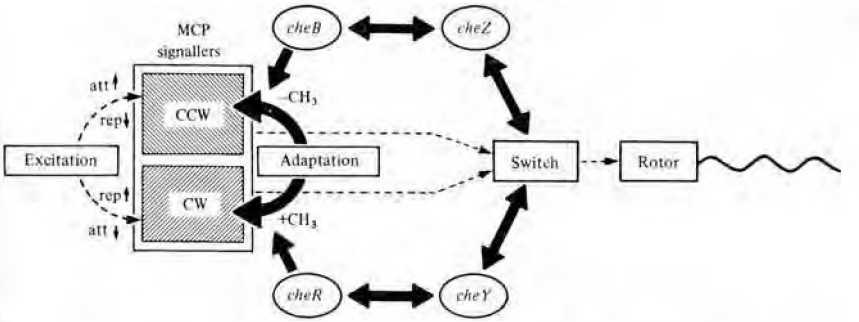

Fig. 5. Information flow during excitation and adaptation. The excitation pathway (dotted lines) is responsible for triggering changes in flagellar rotation in response to chemotactic stimuli. The adaptation system (large arrows) cancels those responses. Both systems appear to act on the MCP signallers in the cytoplasmic membrane. Gene product interactions that could play a role in feedback regulation of the adaptation process have been inferred from genetic studies (Parkinson & Parker, 1979; Parkinson *et al.*, unpublished data).

play a dual role in chemotaxis. They interact with occupied chemoreceptors to generate tumble-modulating signals and then are acted upon by the methylation–demethylation system to cancel or negate those signals to achieve adaptation.

A simple scheme that summarizes the dual function of MCPs in chemotaxis is shown in Fig. 5. This model postulates that the *tar*, *tsr*, and *trg* signallers can each exist in two conformational states, one that causes CCW rotation of the flagella and one that causes CW rotation. The pattern of flagellar rotation thus reflects the relative amount of signaller species in each form. Conversion of a signaller from one rotational state to the other is caused by stimuli, i.e. occupied chemoreceptors, and by adding or removing methyl groups.

Methylation enzymes

Mutants defective in *cheR* function have low spontaneous tumbling rates and can respond to tumble-enhancing stimuli, but do not show adaptation to those stimuli (Parkinson & Revello, 1978; Goy, Springer & Adler, 1978). This behaviour is essentially identical to that of methionine-deprived cells, which are unable to add methyl groups to MCP. *In vivo* studies of *cheR* strains have shown that they are defective in incorporating labelled methyl groups from methionine into the methyl-accepting proteins (Goy *et al.*, 1978). The MCPs present in *cheR* strains are nearly devoid of methyl

groups (Hayashi, Koiwai & Kozuka, 1979) demonstrating that the inability of *cheR* mutants to turn over MCP methyl groups is due to an inability to add methyl groups to the MCP molecules.

Mutants defective in *cheB* function exhibit abnormally high tumbling rates, but are able to respond to certain tumble-suppressing stimuli (Parkinson, 1974, 1976). Usually, *cheB* strains exhibit transient responses to such stimuli; however, when subjected to combinations of stimuli that utilize several MCP species for signalling, *cheB* mutants exhibit prolonged responses indicative of some sort of adaptation defect (Rubik & Koshland, 1978). Methylation assays *in vivo* showed that *cheB* mutants do not turn over MCP methyl groups (Kort *et al.*, 1975; Stock & Koshland, 1978). The MCPs present in these strains appear to be fully methylated, (Hayashi *et al.*, 1979), implying that *cheB* function is required to remove MCP methyl groups.

Methylation assays in permeabilized cells or with crude membrane preparations indicate that wild-type cells contain soluble proteins that catalyse the addition or removal of methyl groups on MCP. Mutants defective in *cheR* function lack the methyltransferase (i.e. methyl-adding) activity (Springer & Koshland, 1977); mutants defective in *cheB* function lack the methylesterase (i.e. methyl-removing) activity (Stock & Koshland, 1978). The steady-state level of MCP methylation observed in wild-type strains is presumably due to a balance between these two enzymatic reactions. In *cheR* and *cheB* strains, where one of these activities is defective, a very low or very high steady-state level of MCP methylation results. The aberrant tumbling rates of *cheR* and *cheB* strains may be caused by the aberrant methylation levels: *cheR* mutants are undermethylated and show predominately CCW flagellar rotation; *cheB* mutants are overmethylated and show predominately CW rotation. These phenotypes are consistent with the data summarized in Fig. 4 which show for example that adaptation to CCW stimuli is accompanied by the addition of methyl groups to the MCPs. Thus methylated MCP is correlated with CW rotation and unmethylated MCP with CCW rotation.

Methylation control

In the absence of chemotactic stimuli there is a slow turnover of methyl groups on the MCPs. The resultant methylation level reflects the steady-state rates of the addition and removal reactions. Upon

excitation, the activity of one or both of these reactions must be altered in order to achieve a net change in methylation level. When adaptation is complete, a new steady-state methylation level is maintained. This level is directly related to the abundance of occupied chemoreceptors; large proportional changes in receptor occupancy upon stimulation elicit correspondingly large changes in the steady-state level of MCP methyl groups.

How does the cell regulate the level of MCP methylation in response to chemotactic stimuli? *A priori*, control could be exerted either by altering the substrate proteins (MCPs) or by altering the methyltransferase or methylesterase enzymes. For example, changes in chemoreceptor occupancy probably cause conformational changes in the cognate signaller species during excitation. These conformational changes might in turn generate new methyl-accepting sites on the MCP molecule or expose previously protected methylated sites. If the methyltransferase and methylesterase activities were substrate-limited, these changes in substrate conformation would lead to new steady-state methylation levels. An alternative means of control would be through modulation of the catalytic activity of the enzymes themselves, rather than their substrates. The evidence discussed below suggests that both of these mechanisms may come into play during the adaptation process.

Following application of a stimulus, only one species of MCP undergoes a *net* change in methylation level. The MCP involved is always the one that is responsible for generating the excitatory signal for a particular stimulus. For example, serine stimuli which are processed by MCPI, the *tsr* product, cause a net change in methylation of only MCPI. This result implies that methylation levels are controlled by substrate conformation. However, such stimuli also cause *transient* methylation changes of non-signalling MCPs (M. S. Springer *et al.*, personal communication). For example, a serine increase causes a brief increase in the level of methyl groups on MCPII, but MCPII methylation returns to pre-stimulus levels upon completion of the adaptation process. This effect could be due to a transient change in the conformation of the MCPII substrate, even though MCPII is not required to process serine signals. Alternatively, this effect could be caused by transient stimulus-elicited changes in the catalytic activities of the methyltransferase and methylesterase enzymes. Several lines of indirect evidence suggest that this latter explanation may be correct.

Genetic studies have shown that both the methyltransferase

(*cheR* product) and the methylesterase (*cheB* product) interact with other chemotaxis components. For example, in interspecies complementation tests, *S. typhimurium* mutants defective in *cheR* or *cheY* activity can only be corrected by supplying *both* of the homologous functions from *E. coli* (DeFranco *et al.*, 1979). Similarly, *both cheB* and *cheZ* function from *E. coli* are needed to complement *Salmonella* mutants lacking either *cheB* or *cheZ* activity. These results suggest that there may be an interaction of some sort between the *cheR* and *cheY* products and between the *cheB* and *cheZ* products. Although these interacting complexes are evidently functionally interchangeable between species, the components of the complexes are not. Thus *cheR* product from *E. coli* can only interact productively with *cheY* product from *E. coli*; the *Salmonella cheY* product does not suffice. These interactions could play a role in modulating activity of the methylation–demethylation enzymes during adaptation. Neither *cheY* nor *cheZ* function is essential to either enzyme activity; however some *cheY* and *cheZ* strains exhibit defects in methylation of MCPs *in vivo*, implying that altered forms of these gene products may interfere with the enzymatic activities of the *cheR* and *cheB* proteins (Kort *et al.*, 1975). Methylesterase activity in extracts of some *cheZ* strains is quite low, whereas it is essentially normal in other *cheZ* strains (Stock & Koshland, 1978).

A summary of gene product interactions involved in excitation and adaptation is shown in Fig. 5. Since the flagellar switch interacts with the *cheY* and *cheZ* products, which in turn interact with the *cheR* and *cheB* products, respectively, it seems possible that the methyltransferase and methylesterase activities are modulated by feedback signals from the switch. The mechanism by which these interactions modulate enzymatic activity is not known, but one possibility is shown in Fig. 6. If the *cheR-cheY* and *cheB-cheZ* products form tight complexes, the switch could modulate enzyme availability by selectively binding one complex or the other. For example, a CCW signal from an MCP, upon interaction with the flagellum, might cause the switch to release *cheY* product and to bind *cheZ* product, thereby activating the methyltransferase and inhibiting the methylesterase. Methylation of the MCPs would proceed until the CCW signal had been cancelled. The switch would then return to its pre-stimulus behaviour and the activity of the two enzymes would return to steady-state values. MCP signals causing CW rotation would activate the methylesterase and inhibit the methyltransferase in an analogous manner.

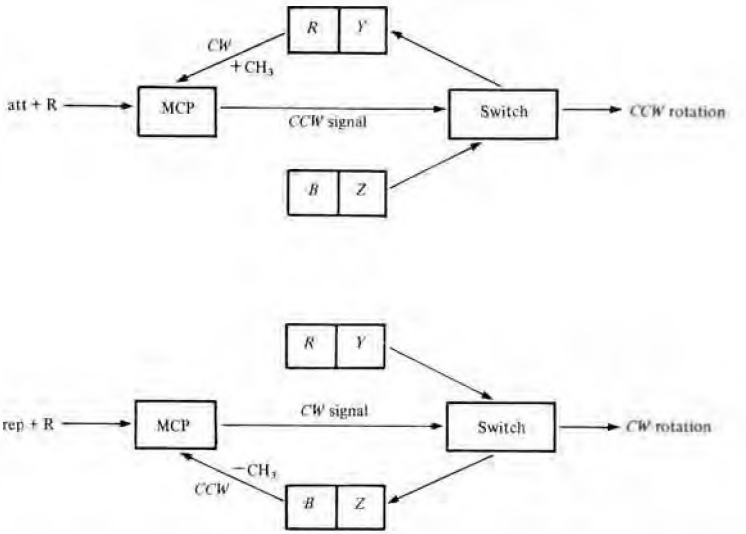

Fig. 6. A model of feedback control of the adaptation machinery. The enzymatic activity of the methyltransferase (*cheR* product) and methylesterase (*cheB* product) may be modulated simply by controlling access to the MCP substrates by means of reversible binding to the flagellar switch.

CONCLUSION

Genetic studies of chemotaxis and motility in *E. coli* and *S. typhimurium* have revealed a large number of functions involved in the excitation and adaptation phases of the chemotactic response. It seems unlikely that additional gene products will be found to have a direct role in these events, although functions essential to cell viability could also be involved. As discussed here, genetic studies of various chemotaxis mutants have contributed to understanding the overall strategy of information processing by the chemotaxis machinery. Identification of the excitatory signal that controls flagellar rotation remains a major unsolved problem in this field. However, biochemical and genetic studies of the adaptation process are rapidly converging on a consistent, detailed view of the role of protein methylation–demethylation events in sensory adaptation. It is not unreasonable to predict that the powerful combination of genetic and biochemical tools that have been brought to bear on this system will soon lead to a molecular description of the entire stimulus transduction machinery in bacteria.

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